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OBSERVATIONS UPON COMPLEMENT-FIXATION IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS.[1]

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In the following contribution I shall give the results of certain experiments upon complement-fixation in tuberculosis that have been made in this laboratory during the past year, and that appear to indicate that in this method we possess a valuable aid in the diagnosis of active tuberculous infection and a guide as to the results of therapeutic measures.

The method of applying the test as detailed in this report is not claimed to be perfect by any means, for further research will undoubtedly result in improvements both in technic and results; but as it has given, in my hands, results closely comparable with those obtained with the Wassermann test in syphilis, it is believed that a description of the method is justified. It is hoped that others, who are more favorably situated for work upon this subject may consider the results obtained with this test encouraging enough to warrant further research, and that, as the result of such research, a method will be evolved that will be practical and easy of application, and that will enable us to diagnose tuberculosis in its earliest stages.

[1] Published with authority of the Surgeon-General, United States Army.

Historical. It would be unprofitable to review here the very numerous attempts that have been made in the past to perfect a method of complement-fixation in tuberculosis that would give uniform and accurate results, and that would be of service in the diagnosis of the disease. Although it was the studies of Wassermann and Bruck upon complement-fixation in tuberculosis that first induced them, together with Neisser, to investigate the possibility of working out such a test in the diagnosis of syphilis, an effort rewarded by the discovery of what is now known as the Wassermann test, up to the present time no method of complement-fixation in the diagnosis of tuberculosis has been generally accepted as of much value by the medical profession.

The researches of Citron appear to demonstrate that after the injection of tuberculin, antibodies are formed capable of binding complement; but the production of these antibodies is transitory, and they soon disappear from the blood serum. Likewise, it has been suggested that during an active tuberculous process complement-binding bodies may be present in the blood serum, but only in small amount, and at varying intervals, while they entirely disappear if the tuberculous process becomes quiescent. Aside from the question of the discovery of a suitable antigen, it has been quite generally held that a complement-fixation test in tuberculosis could never be of practical value because of the variability in the presence of suitable antibodies in the blood serum and in their amount.

However, the consensus of opinion of late appears to be that with a suitable antigen one should be able to devise a complement-fixation test for tuberculosis that would give useful results. This opinion has lead to the study of antigens that might be used in such a test, and for this purpose various substances have been used, as watery and alcoholic extracts of tuberculous organs, either alone or combined with some form of tuberculin; tuberculin alone; tuberculin filtrate; watery extracts or emulsions of the tubercle bacillus; and mixtures of various tuberculins. With the exception of the antigens described by Besredka and by Hammer, no very practical results have been obtained with any of those mentioned.

The antigen recommended by Besredka,[2] consisting of a suspension of bacterial substance derived from dried and ground tubercle bacilli, and that of Hammer,[3] consisting of an alcoholic extract of tuberculous tissue to which has been added a certain amount of old tuberculin, appear to have given very consistent results; but, unfortunately, reactions have occurred with these antigens in other conditions, especially in syphilis, and in a considerable proportion of the cases tested.

[2] Compt. rend. Soc. de biol., 1914, lxxvi, 180.
[3] Münch. med. Wchnschr., 1912, lix, 1750.

In a recent very valuable study by Bronfenbrenner[4] the results are given of experiments made with the antigen of Besredka in the diagnosis, by complement-fixation, of tuberculosis. He found that in active tuberculosis he obtained 93.8 per cent. of positive reactions; in convalescents presenting no symptoms of the disease, 55.5 per cent. of positive results; while in cases strongly suspected of tuberculosis but in which no definite diagnosis has been made, 75 per cent. gave a positive reaction. However, in treated and untreated cases of syphilis he obtained 24 per cent. of positive reactions with this antigen and 8 per cent. of his controls reacted positively. He found that he could separate from Besredka's antigen the substance or substances giving positive reactions with syphilis and other conditions, and that this purified antigen apparently was specific for tuberculosis. In regard to certain negative reactions obtained in undoubted active tuberculosis he states that they suggest that in certain stages of the infection the circulating antibodies apparently disappear from the blood.

Several other investigators have reported very favorable results with Besredka's antigen, notably Inman,[5] who obtained 95 positive reactions in 100 cases of active tuberculosis; and while these reports are very encouraging, the large percentage of syphilities that react positively with this antigen militates against its use as a diagnostic agent. On the other hand, the method described in this paper does not possess this disadvantage, for the antigen employed does not give positive results with the blood serum of syphilitics in the absence of coincident tuberculous infection.

Technic and Material. The general technic of the complement-fixation test for tuberculosis experimented with is that employed in making the Wassermann test in this laboratory, and which I have described fully in previous contributions.[6,7,8]

The *antigen* employed consists of an extract of several strains of the human tubercle bacillus, prepared as follows: The various strains of the bacilli are grown on a liquid medium composed of alkaline bouillon to which a teaspoonful of egg white and egg yolk has been added to each 250 c.c. of the bouillon. After growth is well advanced, an equal amount of 95 per cent. alcohol is added to the culture, and the whole shaken in a shaking machine for twelve hours; the mixture is then allowed to stand in an incubator at 37° C. for twenty-four hours, after which it is again shaken for six hours and filtered through a very fine filter paper or through a Berkefeld or other equally good filter. It appears to make no difference

[4] Arch. Int. Med., 1914, xiv, 786.
[5] Compt. rend. Soc. de biol., 1914, lxxvi, 251.
[6] Craig, C. F., Jour. Exper. Med., 1910, xii, 726.
[7] Craig, C. F., Jour. Infect. Dis., 1911, ix, 213.
[8] Craig, C. F., and Nichols, H. J., Studies of Syphilis, Bull. No. 3, War Dept., Office Surg.-General of Army, 1913, lxxii.

whether the mixture be filtered through a paper or through the Berkefeld so far as antigenic properties are concerned. The filtrates of the strains cultivated are mixed together and the resultant mixture titrated for anticomplementary, hemolytic, and antigenic qualities. The undiluted mixture should be titrated first, as in my experience it is seldom necessary to dilute the mixture.

An antigen may also be prepared by carefully transferring a portion of all of the strains used to a large culture flask, planting each strain far enough apart so that it may be observed whether growth occurs. Generally one or more of the strains transplanted in this manner fail to grow, but this can be disregarded if one works with eight or more strains. When growth has occurred to a considerable extent the alcohol is added, as already described, and the antigen prepared in a similar manner. Antigens made in this way appear to be slightly stronger in antigenic content than when the filtrates of the separately cultivated strains are mixed, but the greater difficulty of cultivating so many strains of the bacillus together more than offsets the slight gain in complement-binding power.

The antigen must be kept in the ice-box except when a small portion is removed for use in the test, as exposure to room temperature, even for a short time, markedly lessens its antigenic properties. It is also necessary that the greatest care be taken that all utensils used in the preparation of the antigen are sterile.

The preparation of a polyvalent antigen for use in this test was suggested by the fact that there are modifications of both the human and bovine types of tubercle bacilli and that some strains of the organisms differ from others in infective power and in cultural reactions. It is probable, indeed, that by adding strains of the bovine bacillus to the antigen, better results will be obtained than when the human strains alone are used, and this subject is now being investigated. It is also believed that a polyvalent antigen prepared by extracting the various strains of the bacillus grown upon solid media, using only the bacterial growth, will probably prove as accurate as the antigen described, and, if, so, it will greatly simplify the method of making the antigen.

A human *hemolytic* system was employed in the test, the amboceptor being obtained by injecting rabbits with human red-blood corpuscles in the usual manner, and the *blood suspension* used consisted of a 1 per cent. suspension of human red-blood corpuscles in normal saline solution. As *complement* guinea-pig blood serum was employed, and this was invariably titrated just before use. The blood sera tested were inactivated at 56° C. for one-half hour before testing, and the final reading of the test was generally made an hour after taking the specimens from the water-bath.

The methods of preparing and titrating the various reagents and the technic of performing the test were the same as in the

Wassermann test as made in this laboratory, and have been fully described elsewhere.[2]

Material. Through the kindly assistance of Colonel Bushnell, Medical Corps, U. S. Army, commanding the U. S. Army General Hospital at Fort Bayard, and Lieutenant Callender, who collected the specimens, I was able to test the blood of 166 patients in that institution suffering from pulmonary tuberculosis. In addition, the blood of 150 syphilities as well as of 100 patients suffering from other diseases was tested, and also 150 normal individuals who were used as controls.

Method of Reading the Results of the Test. The final result of the test was read from an hour to two hours after the last incubation in the water-bath, and this is important, as otherwise some of the positive reactions may become weak or negative. If left in the ice-box over night many of the strongest positive reactions are negative in the morning. Accurate results can only be obtained by reading the test within the time specified.

Early in the work it was determined to estimate the strength of the reaction in tuberculosis in the same manner as the Wassermann test in syphilis, in which the results have been recorded as double-plus (++); plus (+); plus-minus (±); and minus or negative (−). When complete inhibition of hemolysis is obtained the result is recorded as double-plus; when less than 50 per cent. of the blood corpuscles are hemolyzed, as plus; when more than 50 per cent. are hemolyzed, as plus-minus; and if complete hemolysis occurred as minus, or negative. However, it was quickly found that many cases of tuberculosis gave very strong plus reactions, so that it early became evident that a plus reaction with this test was diagnostic of tuberculosis, as it did not occur in other diseases or in healthy individuals. On the other hand it was also found that a plus-minus reaction sometimes occurred both in other diseases and in normal individuals, so that this type of reaction had to be considered as practically negative. In considering the results of the test as here given it should be understood that a positive result includes both double-plus and plus reactions, while a negative includes both plus-minus and minus reactions.

As regards the relative number of double-plus and plus reactions it may be stated that of the 142 cases of pulmonary tuberculosis that reacted to the test, 119 gave a double-plus reaction and 23 a plus reaction; of the 103 cases of active infection reacting, 87 gave a double-plus reaction and 16 a plus reaction; while of the 39 inactive cases of infection, 32 gave a double-plus reaction and 7 a plus reaction. Owing to the percentage of tuberculous cases giving a plus reaction, the test as at present developed requires very careful titra-

[2] Craig, C. F., and Nichols, H. J., Studies of Syphilis, Bull. No. 3, War Dept., Office Surg.-General of Army, 1913, lxxii.

tion of all the reagents and experience in reading the results of hemolytic reactions.

The tests made in this laboratory in the study of this subject may be divided into three groups, as follows: Group 1. Cases in which the diagnosis was pulmonary tuberculosis, either active or inactive. Group 2. Cases in which the diagnosis was of some other disease. Group 3. Normal individuals.

Results of the Test in Group 1. In order to understand the results of the complement-fixation test and their significance in this group it is necessary to briefly describe the classification of the cases tested, which is that followed at the U. S. Army General Hospital at Fort Bayard, New Mexico.

The cases tested were divided into three general classes: Class 1, including incipient cases of tuberculosis; Class 2, moderately advanced cases; and Class 3, far-advanced cases. In addition the cases were classified as *active* and *inactive*, and, again, with reference to the extent of the involvement of lung tissue as *Involvement 1*, indicating a slight lesion extending at most to the volume of one lobe or two half-lobes; *Involvement 2*, slight lesion extending further than *1*, but at most to the volume of two lobes or a severe lesion extending at most to the volume of one lobe; and *Involvement 3* including all lesions which in extent of the parts affected exceeded *Involvement 2*.

The total number of cases of pulmonary tuberculosis tested was 166, of which 142, or 85.5 per cent., gave positive results, and 24, or 14.4 per cent., gave negative results.

The cases were divided as regards the nature of the infection into *active* and *inactive* infections, and the following table gives the results of the test in each class:

TABLE I.—RESULTS OF THE COMPLEMENT-FIXATION TEST IN PULMONARY TUBERCULOSIS WITH REFERENCE TO THE ACTIVITY OF THE INFECTION.

Total cases.	Active.	Inactive.	Positive.	Per cent.	Negative.	Per cent.
166	107	...	103	96.2	4	3.7
	...	59	39	66.1	20	33.8
	107	59	142	85.5	24	14.4

From the foregoing table it is evident that the percentage of positive results obtained with this test in active tuberculous infection was much higher than in the cases classed as inactive, 96.2 per cent. of the active cases giving positive results, while only 66.1 per cent. of the inactive cases reacted to the test. It should be remembered that the classification into active and inactive cases is entirely clinical, and that many of the cases giving a positive result in the inactive class were merely quiescent and may or may not have shown the bacilli in the sputum.

The percentage of positive results in active tuberculous infection of the lungs, *i. e.*, 96.2 per cent., is as high as that obtained in my experience with the Wassermann test in secondary syphilis, for in 1969 cases in that stage of the disease I have obtained positive reactions in 96.1 per cent. of the cases. This speaks very favorably for the accuracy of this test in the diagnosis of active tuberculous infection, and indicates that in this class of cases we may expect as good results with it as are obtained with the Wassermann test in secondary syphilis.

The percentage of positive results in clinically inactive tuberculous infection is practically the same as that I have obtained in latent syphilis with the Wassermann test. In the clinically inactive cases of tuberculosis, 66.1 per cent. gave a positive reaction, while in 1354 cases of latent syphilis I have obtained 69.4 per cent. of positive results.

The importance of the comparatively large percentage of positive results in clinically inactive tuberculosis consists in the demonstration of the fact that the infection in these cases was not really inactive, if we believe that complement-fixing bodies in the blood indicate an activity of the infection. In the past it has been generally accepted that such bodies were not demonstrable in the blood serum unless tuberculosis was clinically active; but the results of this test prove that such bodies can be demonstrated when the disease is inactive clinically. For this reason it would appear that a positive reaction indicates an active focus somewhere in the body and that the test will prove of value in differentiating really cured infections from those which are simply quiescent.

Relation of the Results to Class of Patients Tested. It has been stated that the cases of pulmonary tuberculosis tested were divided into three classes: incipient cases, advanced cases, and far-advanced cases. It was found, as would be expected, that the highest percentage of positive results was obtained in patients in whom the infection was furthest advanced, as is shown in the following table:

TABLE II.—COMPLEMENT-FIXATION IN TUBERCULOSIS IN RELATION TO THE CLASS OF INFECTION.

Class.	Character of infection		Positive reaction.	Per cent. positive.	Negative reaction	Per cent. negative.
	Active.	Inactive.				
1. Incipient cases	1	..	1	100.0	0	0.0
	..	24	14	58.3	10	41.6
Total, 25	1	24	15	60.0	10	40.0
2. Moderately advanced cases	53	..	51	96.2	2	3.7
	..	32	22	68.7	10	31.2
Total, 85	53	32	73	85.8	12	14.1
3. Far advanced cases	53	..	51	96.2	2	3.7
	..	3	3	100.0	0	0.0
Total, 56	53	3	54	96.4	2	3.5

While in the foregoing table the percentage of positive results happens to be the same for active infections in both moderately advanced and far-advanced cases, in the inactive infections the positive percentage is much greater in the far-advanced cases, and in the incipient cases the percentage of negative results is higher than in either the moderately advanced or far-advanced infections.

The relation of the results of the complement-fixation test to the amount of lung involvement is shown in Table III, where the infections are arranged in accordance with the amount of involvement, as previously defined.

TABLE III.—COMPLEMENT-FIXATION IN PULMONARY TUBERCULOSIS IN RELATION TO THE AMOUNT OF LUNG INVOLVEMENT.

Nature of infection.	Involvement.			Positive reaction.	Per cent. positive.	Negative reaction.	Per cent. negative.
	1	2	3				
Active	29	..	..	27	93.1	2	6.8
	..	30	..	30	100.0	0	0.0
	..	..	48	46	95.8	2	4.1
Total, 107	..	..	..	103	96.2	4	3.7
Inactive	43	..	..	27	62.7	16	37.2
	..	14	..	10	71.4	4	28.5
	..	..	2	2	100.0	0	0.0
Total, 59	..	..	..	39	66.1	20	33.8

In general, it will be noted that the greater the amount of lung involvement the greater the percentage of positive reactions, both in the active and inactive infections, and while it may be objected that the number of cases tested is small, I believe that further research will confirm these results.

Results of the Test in Group 2. This group, as stated, included all cases in which the clinical diagnosis was of some disease other than tuberculosis. In all, 250 individuals were tested, of whom 150 were suffering from syphilis and 100 from other diseases, including both acute and chronic conditions.

Of the total number examined, two gave a positive reaction with the test, or 0.8 of 1 per cent. In both positive cases the patients were syphilitic and gave a positive Wassermann reaction; but careful examination revealed lesions of the lungs suggestive of tuberculosis, although no bacilli were demonstrated in the sputum.

That the positive reactions obtained in these two cases giving a positive Wassermann reaction were not due to the syphilitic infection is proved by the fact that of 150 syphilitics tested, all giving a positive Wassermann, not one gave a positive reaction with the antigen employed in the complement-fixation test for tuberculosis here described, a result strongly in contrast with that obtained when Besredka's antigen is used, for with the latter antigen Bronfenbrenner obtained nearly 24 per cent. of positive results in syphilitics in

whom tuberculosis was not suspected. He states, however, that 5 of the cases included in this percentage have since developed symptoms of tuberculosis, which reduces the percentage of positive results in syphilis to 17 per cent.

Of the patients suffering from other diseases, 100 in number, all gave a negative reaction with the test described in this paper, and while the number of cases is comparatively small, their great variety and the uniformly negative results obtained demonstrate, I believe, that this test will not react positively with diseases other than tuberculosis, nor with the blood serum in cases where a tuberculous infection has become encapsulated, for among this number of individuals there must have been many who had encapsulated tuberculous lesions and who would have given a positive reaction with the tuberculin tests.

Results in Group 3. The blood serum of 150 individuals in good health and free from tuberculous infection, so far as could be ascertained, was tested and a negative result was obtained in every instance. While these tests were made upon young, healthy soldiers, I am very sure that had any of the tuberculin tests been applied to the same individuals a considerable proportion of positive results would have been obtained if our conceptions regarding the percentage of individuals who have inactive tuberculous lesions are correct. The fact that the complement-fixation test in so many individuals was negative, speaks strongly in favor of its being a specific test for active tuberculous infection, and indicates that it will not give positive reactions in inactive cases or where the lesions have become encapsulated.

Discussion of Results. I believe that the experiments recorded in this contribution indicate that a complement-fixation test for tuberculosis, capable of giving as good results in the diagnosis and the control of the treatment of the disease as does the Wassermann test in syphilis, can be evolved, and that the test here described approaches very nearly this ideal. While it is unfortunate that so few cases of tuberculosis in the incipient stage could be tested, for it is in this stage of the disease that the test would be of greatest value in diagnosis, the fact that 60 per cent. of the cases that were tested in the incipient stage gave a positive reaction, although all were recorded as inactive clinically, speaks eloquently for the accuracy of the test in the diagnosis of early cases.

The results of the test also demonstrate that while a patient may have a clinically inactive tuberculous infection, the blood often gives a positive complement-fixation reaction, and this fact should prove of considerable value in the control of the treatment of the disease. While it would be premature to argue that a negative complement-fixation reaction in clinically inactive cases of tuberculosis means that the infection is cured, it is quite evident that in many cases proved to be inactive clinically a positive reaction *is*

obtained, and this certainly would appear to indicate that there is still an active focus present, although no symptoms can be detected. On the other hand, where a negative reaction is repeatedly obtained and no symptoms indicating the presence of an active infection are present, it would appear to be reasonable to conclude that the infection has disappeared. However this may be, I am forced to conclude, as the result of these experiments, that a positive result with the complement-fixation test described, indicates the presence of an active tuberculous infection, even though the disease may be clinically inactive, and that as long as the test remains positive the patient cannot be said to be cured. This conclusion is based upon the uniformly negative results of the test in healthy individuals, the large percentage of positive results obtained in active tuberculosis, and the comparatively large percentage of positive results in clinically inactive cases of the disease.

The strength of the reaction has been found to vary from day to day in some cases, a strongly positive reaction alternating with a plus-minus or even a negative reaction. This variation in the strength of the reaction I have shown to[10] be true of the complement-fixation test in syphilis; and more recently, Irons and Nicolls[11] have shown the same to be true of complement-fixation in gonorrheal infections. Therefore, in this test, as in complement-fixation in the other diseases mentioned, a single negative reaction is of no value in excluding infection. Repeated tests should be made in all cases in which tuberculosis is suspected, and a negative report should not be made until several tests have been performed.

CONCLUSIONS. The following conclusions appear to be justified from the results of the complement-fixation test for tuberculosis reported in this paper:

1. Complement-binding antibodies are present in the blood serum of both active and clinically inactive tuberculous infections.

2. A polyvalent antigen prepared from several strains of the human tubercle bacillus has been found to give excellent results in complement-fixation for tuberculosis.

3. With the test described, complement-fixation gave a positive reaction in 96.2 per cent. of cases of active tuberculosis and in 66.1 per cent. of the cases of clinically inactive tuberculosis.

4. The test was negative in normal individuals and in patients suffering from other diseases with the exception of two patients infected with syphilis in whom symptoms of a coincident tuberculous infection was also present.

5. The test does not give positive results with the blood serum of syphilitics in whom there is no coincident tuberculous infection.

6. The reaction, when positive, is specific and apparently indicates.

[10] Craig, C. F., Jour. Amer. Med. Assn., 1914, lxii. 1232.
[11] Jour. Infect. Dis., 1915, xvi, 303.

the presence of an active tuberculous focus, although there may be no symptoms of the disease present.

7. Positive results are obtained in a large percentage (66 per cent.) of clinically inactive cases of pulmonary tuberculosis, and such a result indicates that though it may be quiescent the infection has not disappeared.

8. The results obtained with the test described are practically as good as those obtained with the Wassermann test for syphilis.

Of course, much more work is needed before this, or any other method of diagnosing tuberculosis by complement-fixation is placed upon a thoroughly practical basis and is generally accepted as a routine diagnostic measure, but it is confidently believed that continued work upon this subject will result eventually in the perfection of a test that will be fully as useful in the diagnosis and control of the treatment of tuberculosis as is the Wassermann test in syphilis.

I desire here to express my thanks to Doctor Edward R. Baldwin for seven strains of the human tubercle bacillus kindly furnished me from the Saranac Lake Laboratory, and to Dr. Simon Flexner for three strains of the bacillus from the Rockefeller Institute. The antigens used in my experiments were prepared from six of the strains furnished by Dr. Baldwin and one of the strains furnished by Dr. Flexner.

LANDRY'S PARALYSIS: REPORT OF A CASE WITH NECROPSY AND HISTOPATHOLOGICAL FINDINGS.[1]

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Many cases have been reported since Landry first described a disease characterized by a rapidly ascending paralysis passing from the lower to the upper extremities, thence to the cranial nerves, causing various paralyses, and ending fatally in the course of six or seven days with involvement of the vagus and respiratory failure. The pathological changes in the early cases were negative. This may have been due to the insufficient method of examination in part, but even in later reports very few changes have been found at times.

In the literature of this subject, which is considerable since Landry, in 1859, described his case, we find various lesions recorded in which either polyneuritis or spinal cord or bulbar inflammatory involvement has been present, or all in combination. Leyden and

[1] Read before the Americal Neurological Society, New York City, May 8, 1915.